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New Approaches To The Labeling Of Estrogens Useful For PET

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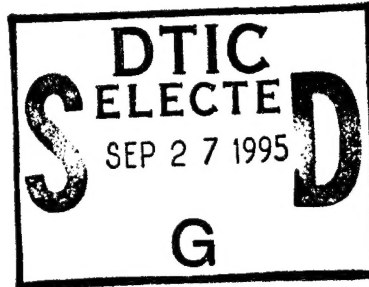
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Progress: During this year, research has focused on understanding the chemistry of methyl hypofluorite (MeOF). MeOF is the only source of the novel electrophilic methoxylum ion species MeO⁺ and has been shown to react readily with C-C double bonds. Quick incorporation of C-11 with the help of MeOF will allow radiolabeling of steroids useful for positron emission tomography (PET) to disclose the location of tumor lesions and metastases with a lower dose of radiation to the patient. An estradiol derivative, 16 α -methoxy estradiol, was selected as the target molecule and synthesized. Radiolabeling of the estradiol derivative with the use of [C-11]CH₃OF was attempted, but yields were low. One of the goals for the following year is to increase this yield. Yields are being optimized by replacing the reaction solvent with radical scavenging chloroform. During the next year, the investigator will optimize radiochemical reactions for the target steroidal molecules and probe their binding affinities in tumor sites.

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New Approaches To The Labeling Of Estrogens Useful For PET

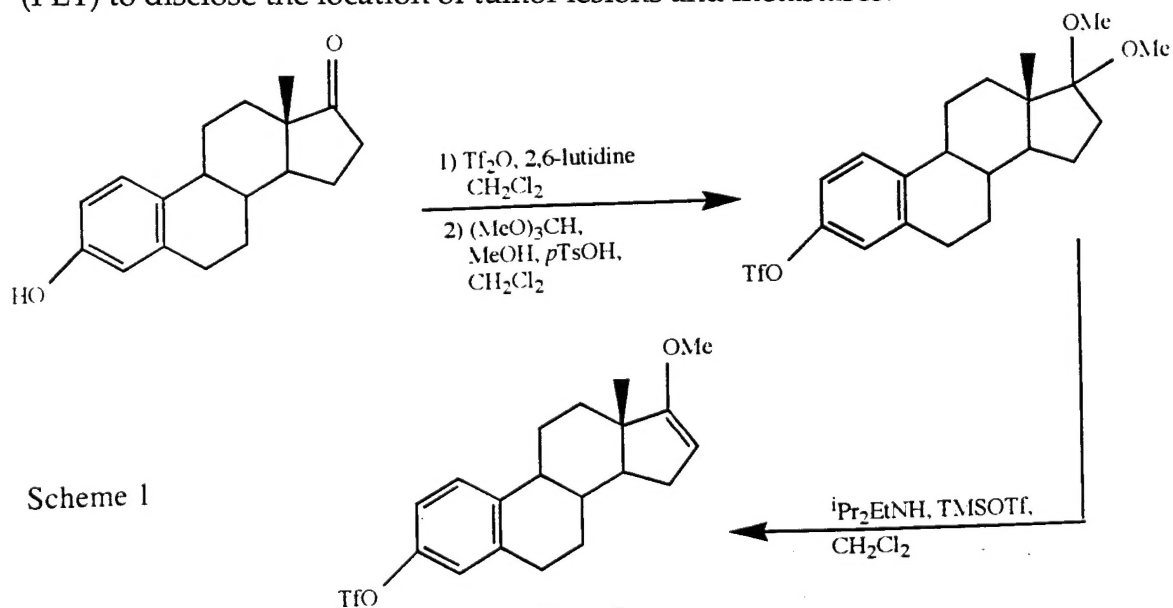
Introduction

The first year of Army funding has coincided with my second year as a graduate student at Washington University in St. Louis, Missouri. Progress toward my doctoral degree in nuclear chemistry this past year includes fulfillment of required courses and laboratory teaching as well as successful completion of all candidacy exams. Passing both the written and oral candidacy exam allows me to continue in the doctoral program in nuclear chemistry. I will obtain a Master's Degree in August 1995 as a result of fulfilling these requirements.

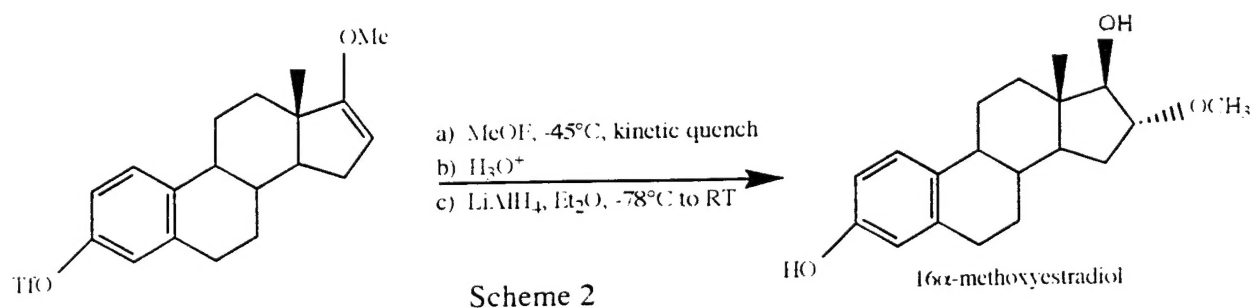
Research goals for the past year focused on understanding the chemistry of methyl hypofluorite (MeOF) and its application to radiolabeling estrogens with C-11. MeOF is the only source of the novel electrophilic methoxylium ion species "MeO⁺" and has been shown to react readily with C-C double bonds¹ and various types of methyl enol ethers.² Quick incorporation of C-11 with the help of MeOF will allow radiolabeling of steroids useful for PET.

Experimental Results and Discussion

The main objective was to synthesize an estrogen analogue that has a high binding affinity for the estrogen receptor found in estrogen receptor positive breast tumors.³ C-11 radiolabeling could then be obtained through the use of [C-11]CH₃OF. This would enable the use of positron emission tomography (PET) to disclose the location of tumor lesions and metastases.



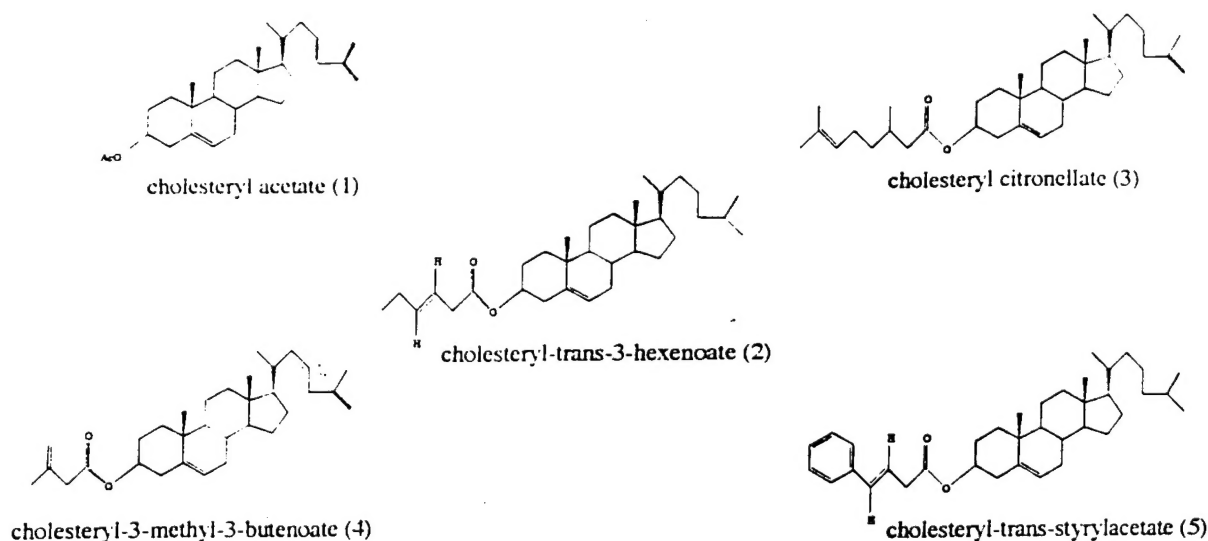
Searching for a biologically interesting target molecule led to the selection of 16 α -methoxy estradiol. The initial substrate synthesized in route to the target molecule was the 17-methylenolether of estrone protected at the 3 position with triflate (scheme 1). Upon reaction with methyl hypofluorite and H₃O⁺, the 16 α -methoxy-3-triflate estrone was obtained. Reduction and deprotection with LiAlH₄ produced the estradiol derivative with a yield of ~10% (scheme 2). This estradiol derivative is expected to be less lipophilic than estradiol based on its calculated log P values (2.86 and 4.63 respectively). Lower lipophilicity is advantageous for it reduces non-specific binding. Binding studies will be done to determine the affinity for the estrogen receptor positive target tissue.



Radiolabeling of the estradiol derivative is accomplished with the use of [C-11]CH₃OF. This reaction is being optimized as the yields are currently low. This work was presented at the National American Chemical Society Meeting in August 1994 and the abstract can be found in Appendix 1.

Desiring to better understand the chemistry of MeOF, a model compound was sought to probe its reactivity. Cholesterol was chosen because it has a rigid steroidal skeleton and internal double bond. Counsell and coworkers have reported radioiodinated esters of cholesterol as potentially useful tumor imaging agents.^{4,5,6} Carbon-11 labeled cholesterol esters would induce a lower radiation dose on the patient (due to the shortened half-life) and would allow for more rapid imaging. With this in mind, various ester linkages each containing one double bond were attached to cholesterol at the 3 position. Therefore, each cholesterol analogue contained 2 double bonds allowing an investigation in to which one MeOF would prefer. The objective was to make the external double bond more reactive than the internal double bond. This would allow the molecule to be radiolabeled on the ester linkage with the help of [C-11]CH₃OF.

Rozen and coworkers have probed the reactivity of methyl hypofluorite with olefins.¹ The more electron-rich double bonds were found to react preferentially with MeOF. Cholesterol acetate (1) which contains an internal tertiary double bond reacted with MeOF to produce 5 α -fluoro-6 β -methoxy cholestanol acetate with a yield of 20%. Desiring to probe the reactivity of the external double bond in the presence of the internal double bond, various cholesterol esters containing a secondary double bond (2), tertiary double bond (3), terminal tertiary double bond (4), and a benzylic double bond (5) were synthesized and reacted with MeOF.



Reacting cholesterol-*trans*-3-hexenoate (2) with MeOF only resulted in addition of MeOF to the internal double bond as identified by NMR. This was as expected for the internal tertiary double bond is more electron rich than the external secondary double bond. The tertiary internal double bond of cholesterol had provided a more stable cation intermediate which has been shown to occur during the addition of MeOF.² A solubility problem was noticed during the reaction of MeOF and the steroid. Methyl hypofluorite is generated in acetonitrile at -45°C, but the steroidal substrates are not soluble in acetonitrile. The solubility problem has been solved by adding the methyl hypofluorite/acetonitrile to the substrate dissolved in methylene chloride providing a 2:1 solution of acetonitrile and methylene chloride. The cholesterol-citronellate (3) molecule exposed MeOF to two tertiary double bonds, however, the internal double bond provided a less hindered environment. No external addition of MeOF was observed.

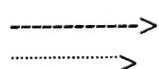
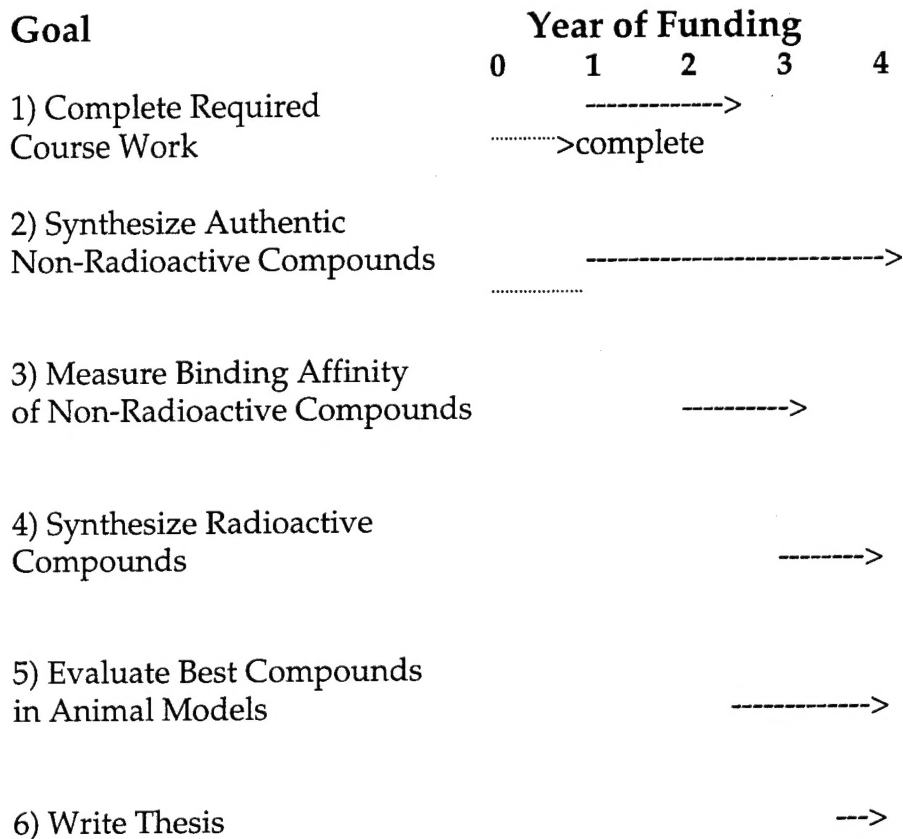
The rigidity of the steroidal skeleton provides an unhindered attack path for MeOF. It was desired to make the external double bond less hindered to increase its reactivity. The chosen molecule was cholesteryl-3-methyl-3-butenolate (4) which possesses a terminal tertiary double bond. When reacted with MeOF, NMR showed that the internal double bond was preferred to the external double bond by a ratio of 4:3. The product containing the internal double bond was isolated from the reaction mixture and characterized by ^1H NMR and high resolution mass spectrometry.

With the benzylic external double bond (5), addition of MeOF preferred the external double bond to the internal double bond 4:1. The electron rich benzylic double bond proved to be more reactive than the others. The benzylic compound is also advantageous for it is UV active. This allows the reaction to be purified and monitored by HPLC using a UV detector at $\lambda=254$ nm. HPLC conditions are currently being determined for the reaction of cholesteryl-trans-styrylacetate with methyl hypofluorite. Soon to follow will be the reaction with $[\text{C-11}]\text{CH}_3\text{OF}$ to probe the preference for the internal double bond to the external benzylic double bond at the tracer level.

Conclusion: Future Plans

This past year has provided valuable experience for working with methyl hypofluorite. Unseen problems arose when the chemistry of methyl hypofluorite was combined with steroids. Difficulties with solubility have been solved and the obstacle of purification continues to be rectified. Yields are being optimized by replacing the reaction solvent, currently methylene chloride, with radical scavenging chloroform. These problems have been counteracted as they have arisen providing the development of problem solving strategies needed in the research field. I look forward to optimizing the syntheses of the non-radioactive molecules in the coming year and hopefully to carry out binding studies and radiochemical syntheses. This will be ahead of my original schedule. A time line for the statement of work follows.

Time Line for Statement of Work



represents original time estimate
 represents work completed

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Appendix 1

Abstract of Oral Presented at the National American Chemical Society Meeting
in Washington, DC, August 1994.

42. CHEMISTRY AND RADIOCHEMISTRY OF NO-CARRIER ADDED $^{11}\text{CH}_3\text{OF}$
Stephanie D. Jonson, Thomas A. Bonasera, Timothy J. McCarthy, Michael J.
Welch, Mallinckrodt Institute of Radiology, Washington University School of
Medicine, St. Louis, MO, 63110.

Methyl hypofluorite has been reported to undergo reactions with various enol ethers to furnish α -methoxy ketones. This new synthetic intermediate is the only source of methoxylum ion " MeO^+ " (S. Rozen, E. Mishani, and M. Kol. *J. Am. Chem. Soc.*, Vol. 114, No. 20, 1992) and has allowed for several novel reactions to be developed. We have developed $^{11}\text{CH}_3\text{OF}$ and synthesized model α -methoxy ketones. The use of this radioactive reagent allows for the introduction of carbon-11 into various substrates. Methyl hypofluorite is being utilized to produce radioactive and non-radioactive novel steroids. The approaches to the design of these novel steroidal structures will be described.